Table VI—Summary of Partial Saturation Experiments and Best-Fit Analysis for Aged Pellets in 5% Sodium Cholate and 0.1 *M* Phosphate Buffer at pH 8.00

C_s[a], mg/ml	Revolutions per Minute	Best-Fit Analysis C_s, mg/ml	$P \times 10^4$, cm/sec	$D \times 10^6$, cm²/sec
1.73	20	1.75	1.39	1.26
1.73	50			
1.72	150			
1.73	450			

[a] From extrapolation of partial saturation data.

Table VII—Summary of Partial Saturation Experiments and Best-Fit Analysis for Soaked Pellets in 5% Sodium Cholate and 0.1 *M* Phosphate Buffer at pH 8.00

C_s[a], mg/ml	Revolutions per Minute	Best-Fit Analysis C_s, mg/ml	$P \times 10^4$, cm/sec	$D \times 10^6$, cm²/sec
1.40	20	1.41	0.39	1.26
1.40	50			
1.42	150			
1.42	450			

[a] From extrapolation of partial saturation data.

similar, leaving the pellet at 35° and ambient humidity for 24 hr may effectively dehydrate the outer surface layer, thereby making the pellet anhydrous. After initial dissolution removes the outer layer, dissolution again is controlled by the more slowly dissolving monohydrate layers.

Another possible explanation for this dissolution pattern is that pellet dehydration is not just a phenomenon of the outer surface layer but that the internal portions of the pellet also dehydrate. When the pellet contacts the dissolution medium, the more rapidly dissolving anhydrous material is removed from the surface. Dissolution from the internal portions of the pellet now is controlled by both anhydrous cholesterol and cholesterol monohydrate. The deeper the dissolution goes into the pellet, the less anhydrous material is present. As the curvature becomes greater, more cholesterol monohydrate probably is exposed; thus, dissolution slows and begins to approach the dissolution rate of a normal pellet.

As shown in Fig. 4, pellets exposed to ambient humidity for 24 hr at 35° and then soaked in double-distilled water at 37° for 24 hr showed linear dissolution, which indicates that soaking of an aged pellet effectively rehydrates the pellet. This rehydration is seen in the fact that the solubilities for the soaked and normal pellets are similar. However, the reformation of the monohydrate is probably not to the original state, perhaps because of precipitation of cholesterol onto the pellet surface. The newly formed crystals might be another polymorph of cholesterol monohydrate or they might be more perfect crystals (fewer defects) with fewer effective sites available for dissolution. The lower dissolution rate seen for the soaked pellet can be accounted for by either explanation. If dissolution of the soaked pellet is studied for a long period (~24 hr), a second linear portion is seen in the dissolution profile. Its slope is closer to that of the normal pellet.

CONCLUSION

From this analysis, the need for a uniform experimental procedure can be seen. If investigators hope to reproduce results within their own laboratories as well as reproduce other investigators' findings, standard experimental procedures must be defined and used.

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High-Pressure Liquid Chromatographic Analysis of Sennosides A and B Purgative Drugs

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Received January 21, 1980, from the *Pharmaceutical Analysis Unit, Department of Pharmaceutical Chemistry, University of Ife, Ile-Ife, Nigeria.* Accepted for publication September 10, 1980.

Abstract □ Procedures are described for the analysis of the main anthraquinone glycosides of senna powder, senna fruit tablets, and sennoside tablets by high-pressure liquid chromatography (HPLC). In one HPLC analysis, TLC was used to separate the glycosides prior to elution on a strong anion-exchange column with 0.1 *M* ammonium nitrate solution (pH 9.0) as the mobile phase. In another HPLC analysis, separation was effected using a weak anion-exchange column with 0.1 *M* ammonium nitrate solution (pH 5.7) as the mobile phase.

Keyphrases □ Sennosides A and B—high-pressure liquid chromatographic analysis, separation of isomers from crude drug and pharmaceutical preparations □ Anthraquinone glycosides—high-pressure liquid chromatographic assay, separation of isomeric sennosides A and B from crude drug and pharmaceutical preparations □ High-pressure liquid chromatography—analysis, anthraquinone glycosides, separation of isomeric sennosides A and B from crude drug and pharmaceutical preparations □ Cathartics—anthraquinone glycosides, isomeric sennosides A and B, high-pressure liquid chromatographic analysis

The pods and leaves of senna as well as the pharmaceutical preparations containing sennosides A and B are widely used in medicine because of their laxative properties. An accurate, simple, and easy method is needed for estimation of sennosides A and B individually.

The most commonly used approach involves removal

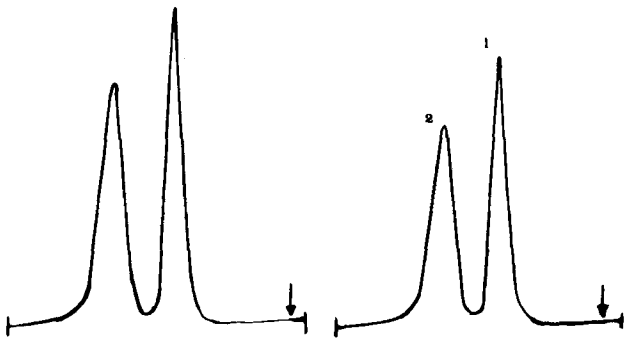

Figure 1—*Chromatogram of sennosides A (2) and B (1). The column was 600 × 2 mm i.d. (DuPont), strong anion exchange (30–40 μm). The mobile phase was ammonium nitrate solution (0.1 M, pH 9.0). UV detection was at 254 nm.*

of free aglycones by partitioning into an organic layer, hydrolysis and oxidation of the glycosides into aglycones and sugars, formation of colored derivatives by the action of aqueous alkali on the liberated aglycones, and absorption spectrophotometry (1–6). This procedure is not specific since it does not distinguish between the isomeric sennosides A and B but gives only a total sennoside content. Moreover, the procedure is ineffective since bianthrones such as the sennosides are not oxidized completely to anthraquinones (7, 8). The presence of several bianthrones in senna, rhubarb, cascara, and frangula suggested the need for a new assay.

The low concentrations of highly labile glycosides in their pharmacologically active forms in purgative drugs make liquid chromatography at ambient temperatures an attractive choice. Application of high-pressure liquid chromatography (HPLC) to the detection and separation of free and combined anthraquinone compounds has been reported (9–14), but HPLC has not been extended to the quantitation of anthraquinone glycosides in drugs and pharmaceutical preparations. This work attempted such a quantitation.

EXPERIMENTAL

Materials—The authentic glycosides used were sennosides A and B. The drugs analyzed include powdered senna pods, senna fruit tablets[1] (each tablet contained sennosides A and B equivalent to 7.5 mg of sennoside B per tablet), and sennoside tablets[2] (each tablet contained 12 mg of sennosides A and B as calcium salts).

The HPLC eluates were monitored by UV detection at 254 nm. The strong anion-exchange columns used were 600 × 2 mm i.d., 30–40-μm particle size[3], and 125 × 4 mm i.d., 10-μm particle size[4]. The weak anion-exchange column was 600 × 2 mm i.d., 30–40-μm particle size[5]. An electronic integrator[6] was used for peak area measurement.

Silica gel was used for TLC. Precoated plastic sheets of silica gel (0.25 mm) containing a fluorescent indicator for UV detection at 254 nm also were used.

n-Propanol, ethyl acetate, ammonium hydroxide, nitric acid, and ethanol were analytical reagent grade. Aqueous reagents were prepared in deionized water, and all glassware was scrupulously cleaned.

TLC—The reference solution was prepared by dissolving sennoside A (~2 mg, accurately weighed) in the TLC mobile phase (1 ml) with gentle warming to effect complete dissolution. A reference solution of sennoside B was prepared similarly.

The test solution of senna powder, senna fruit tablets, or sennoside tablets was prepared by putting the powdered drug (~0.4 g of senna powder or senna fruit tablet powder or 0.2 g of sennoside tablet powder, accurately weighed) in a round-bottom flask and adding 5 ml of ethanol–water (50:50 v/v). The flask was weighed with its contents, a reflux condenser was attached, and the flask was heated in a boiling water bath for 15 min. Then the flask was cooled and adjusted to the original weight with ethanol (50% in water), and the contents were transferred to a centrifuge tube and centrifuged. The clear supernate was used for TLC spotting.

Aliquots (10 μl) of each standard and test solution were applied to the same chromatoplate, allowed to dry, and developed over a path of ~10 cm using *n*-propanol–ethyl acetate–water (40:40:30 v/v/v) as the developing solvent. The solvent was allowed to evaporate at room temperature, and the chromatoplate was examined under UV light to determine the spots corresponding to those of the standards.

HPLC of TLC Spots—Ten TLC spots from each authentic sennoside

[1] Senokot tablets, Reckitt and Collman.
[2] Pursennid tablets, Sandoz Products Ltd.
[3] Zipax.
[4] Partisil.
[5] AL Pellionex.
[6] Minigrator.

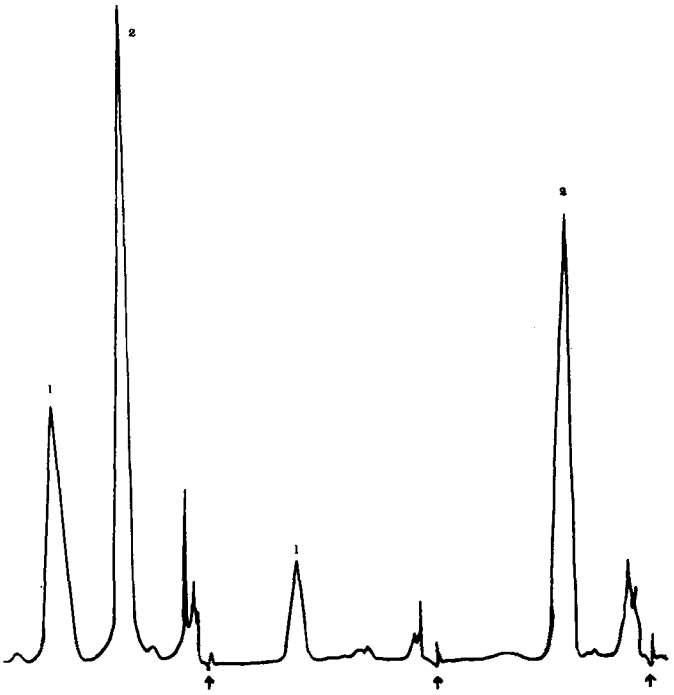

Figure 2—*Chromatogram of sennosides A (2) and B (1). The column was 125 × 4 mm i.d. (Partisil), strong anion exchange (10 μm). The mobile phase was ammonium nitrate solution (0.1 M, pH 7.5) and 15% ethanol. UV detection was at 254 nm.*

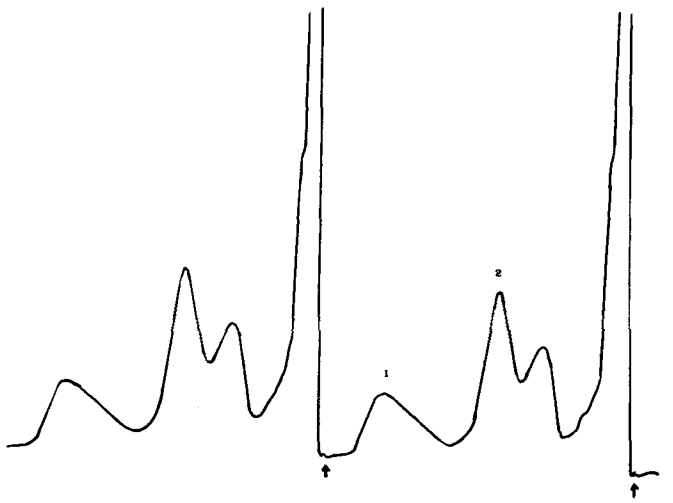

Figure 3—*Chromatogram of the aqueous extract of senna. The column was 600 × 2 mm i.d. (AL Pellionex), weak anion exchange (30–40 μm). The mobile phase was ammonium nitrate solution (0.1 M, pH 5.7). UV detection was at 254 nm. Key: 1, sennoside B; and 2, sennoside A.*

Table I—Sennosides A and B Content [a] of Purgative Drugs as Determined by HPLC

Sample	Sennoside A Content, mg (SAX[b])	Sennoside B Content, mg		Label (Colorimetric Assay)
		SAX[b]	WAX[c]	
Senna (per gram)	14.2	13.9	14.3	28.6
Senna fruit tablets (per tablet)	3.5	3.3	3.62	7.5
Sennoside tablets (per tablet)	5.9	5.8	6.05	12.0

[a] Mean of three determinations. [b] SAX = strong anion-exchange column (30–40 μm) with 0.1 *M* ammonium nitrate solution (pH 9.0) as the mobile phase. [c] WAX = weak anion-exchange column (30–40 μm) with 0.1 *M* ammonium nitrate solution (pH 5.7) as the mobile phase.

spot were extracted with the HPLC mobile phase (1 ml), and a 10-μl portion was injected into the chromatograph and eluted under the following conditions. Column 1 was Zipax, strong anion exchange (30–40 μm), with a mobile phase of 0.1 *M* ammonium nitrate solution (pH 9.0). Column 2 was Partisil, strong anion exchange (10 μm), with a mobile phase of 0.1 *M* ammonium nitrate solution (pH 7.5) and 15% ethanol. The flow rate was 1 ml/min. UV detection was at 254 nm, and attenuation was set at ×16.

Ten TLC spots of the crude senna drug corresponding to sennoside A were extracted with the HPLC mobile phase (1 ml) and eluted under the same experimental conditions as for the authentic sennosides.

Similarly, 10 TLC spots corresponding to sennoside B in the crude senna drug were extracted with the HPLC mobile phase (1 ml) and eluted under the same experimental conditions as for the authentic sennosides. The sennosides A and B content of senna fruit tablets and sennoside tablets also was determined using the same experimental procedure and conditions as outlined for crude senna drug.

Direct HPLC Analysis—Sennosides A and B were dissolved in 0.1 *M* ammonium nitrate (pH 5.7) containing a trace of ammonia solution to give a concentration of ~1 mg/ml each. Aliquots (10 μl) were injected into the chromatograph under the following conditions. The column was AL Pellionex weak anion exchange (30–40 μm), with a mobile phase of 0.1 *M* ammonium nitrate (pH 5.7) at a flow rate of 1 ml/min. UV detection was at 254 nm, and attenuation was set at ×16.

Senna pods or senna fruit tablets (~0.5 g of each drug powder, accurately weighed) was placed in a round-bottom flask with deionized water (10 ml). After the flask was weighed with its contents, it was refluxed in a boiling water bath for 15 min, cooled, and adjusted to the original weight with deionized water. The aqueous extract was centrifuged, and portions of the clear supernate (10 μl) were chromatographed or were mixed with the authentic sennoside solutions and then chromatographed. The same elution conditions were used as for the standard solutions.

In the assay of sennoside tablets, one tablet was ground to a fine powder and then triturated with the mobile phase (5 ml). The suspension obtained was centrifuged and 10-μl portions of the clear supernate were chromatographed or were mixed with the authentic sennoside solutions and then chromatographed under the same conditions used for the standard solutions.

RESULTS AND DISCUSSION

Preliminary investigations showed that the use of different solvents for extraction did not affect the yield or form of the sennosides. This finding is in agreement with the observation of previous workers (15, 16) who found that hot water or water containing up to 70% methanol, ethanol, or acetone effects extraction of most glycosidal contents in a drug–percolate ratio of 1:6. Comparison of the high-pressure liquid chromatograms of the drug extracts containing authentic sennosides with those that did not confirmed the presence of the sennosides in the drugs.

Variations of the HPLC elution parameters (pH and ionic strength)

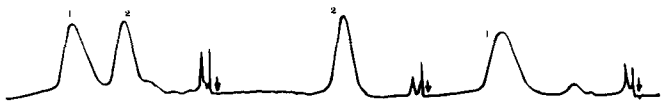

Figure 4—*Chromatogram of sennosides A (2) and B (1). The column was 600 × 2 mm i.d. (AL Pellionex), weak anion exchange (30–40 μm). The mobile phase was ammonium nitrate solution (0.1* M, *pH 5.7). UV detection was at 254 nm.*

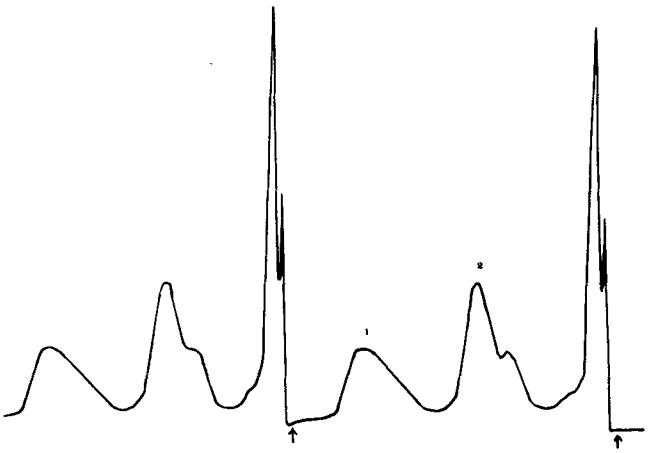

Figure 5—*Chromatogram of the ammonium nitrate extract of sennoside tablets. The column was 600 × 2 mm i.d. (AL Pellionex), weak anion exchange (30–40 μm). The mobile phase was ammonium nitrate solution (0.1* M, *pH 5.7). UV detection was at 254 nm. Key: 1, sennoside B; and 2, sennoside A.*

showed that the optimum conditions for the elutions of sennosides A and B in the two strong anion exchangers included 0.1 *M* ammonium nitrate (pH 9.0) in the larger particle chromedium and 0.1 *M* ammonium nitrate (pH 5.7) containing 15% ethanol in the 10-μm chromedium. At the optimum operational conditions, pure sennosides A and B had symmetrical peaks with good peak width and retention times, and they were well separated from each other. However, the resolution obtained on the larger particle chromedium was better than that obtained on the smaller particle chromedium (Figs. 1 and 2). Ethanol had to be added to the ammonium nitrate mobile phase to obtain elution of the glycosides on the 10-μm ion exchanger within a reasonable time. Alcohol molecules probably block the active adsorption sites on the chromedium, resulting in faster elution of the glycoside molecules.

The chromatogram obtained for senna fruit tablets was similar to that for senna pods on both the strong and weak anion-exchange columns (Fig. 3). This result was expected since senna fruit tablets are made from powdered senna pods. It was anticipated that the 10-μm strong anion exchange chromedium would produce better resolution than the larger particle chromedium (30–40 μm) and that the resolution might free the sennosides from unwanted matter. However, both strong anion-exchange systems, at the optimum elution conditions, did not achieve this end, making direct quantitation of the sennosides A and B content of the drug extracts impossible. However, elutions on the strong anion-exchange column (30–40 μm) with ammonium nitrate (pH 9.0) as the mobile phase gave improved separation of peaks with a decrease in the ionic strength of the mobile phase. The chromatogram of a 0.02 *M* ammonium nitrate solution showed that sennoside A was completely separated from all other peaks, although the retention time was very long (48 min) and the peak width was broad (3 cm). Therefore, direct HPLC quantitation of sennoside A in the crude purgative drug and pharmaceutical preparations might be possible using solvent programming.

TLC of pure sennoside A showed a major spot of R_f 0.31 and a minor spot of R_f 0.51 due to an impurity. TLC of pure sennoside B showed a major spot of R_f 0.16 and two minor spots of R_f 0.34 and 0.048 due to impurities (6, 8). Sennosides A and B in the drug samples were well separated by TLC. The results of the assay based on HPLC of the TLC spots are shown in Table I.

In the direct HPLC elutions of the aqueous extracts of senna pods, senna fruit tablets, and sennoside tablets using the weak anion-exchange column, only sennoside B was well separated from all other components of the drug constituents. Thus, it was possible to estimate only the sennoside B content of the drug by comparison of the peak area with that of authentic sennoside B solution of known concentration (Table I and Figs. 3–5).

The ion-exchange chromatographic separation of the sennosides from the other drug constituents is based on the presence of a carboxylic acid functional group on the sennoside molecules. Since sennosides A and B have the same functional groups and the same molecular configuration, the separation of one from the other on the ion-exchange column must include some other phenomenon (*e.g.*, hydrogen bonding, polar interaction, adsorption, or van der Waals forces) in addition to the reversible exchange of ions between the solute and the electrolyte gel.

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Dosage Form Design for Improvement of Bioavailability of Levodopa IV: Possible Causes of Low Bioavailability of Oral Levodopa in Dogs

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Received April 11, 1980, from the *Product Development Laboratories, Sankyo Company, Ltd., 1-2-58, Hiromachi, Shinagawa, Tokyo, Japan.* Accepted for publication September 30, 1980.

Abstract □ Several potential mechanisms for reduced levodopa bioavailability following oral administration to dogs and humans were investigated by studying the influence of the administration route on plasma levodopa levels after intravenous, hepatoportal, and duodenal administrations to dogs. The observed average areas under the plasma concentration–time curves (*AUC*) of levodopa following hepatoportal injection and intravenous injection were virtually identical; but following duodenal administration a decrease in the *AUC* of levodopa was observed with a concomitant increase in the *AUC* of total dopamine. The possible involvement of intestinal microorganisms in levodopa metabolism was explored in dogs that had been administered a combination of paromomycin and kanamycin to reduce intestinal microflora. Similar patterns of plasma level profiles and urinary excretion were observed between control and treated dogs. As measured by the release of [^{14}C]carbon dioxide from [^{14}C]levodopa, the distribution of levodopa decarboxylase enzyme activity in various parts of the intestine was studied in homogenates prepared from isolated intestinal segments of the duodenum and upper, middle, and lower parts of the jejunum and ileum. The jejunum showed the highest decarboxylase activity followed by the ileum and duodenum. These data indicate that the reduced bioavailability of orally administered levodopa occurs as a result of metabolism by levodopa decarboxylase enzyme in the gut wall.

Keyphrases □ Levodopa—bioavailability, effect of administration route, metabolism, intestinal microorganisms, levodopa decarboxylase □ Bioavailability—levodopa, effect of administration route □ Antiparkinsonian agents—levodopa, effect of administration route on bioavailability

Previous studies (1, 2) reported that, based on the measurement of levodopa and its metabolites recovered in the urine, the total amount absorbed, including levodopa metabolites, was 80–90% of the administered dose. However, the measurements of plasma levodopa concentrations after intravenous and oral administrations indicated that 20–40% of the administered dose reached the fluids of distribution intact.

The present study with levodopa was carried out in dogs to elucidate the mechanisms responsible for the low bioavailability of orally administered levodopa.

EXPERIMENTAL

Influence of Administration Route on Plasma Levodopa Levels and Its Metabolites in Dogs—Nine healthy male beagle dogs, 10.5–13.2 kg, were fasted for ~16 hr and divided into three groups. They were anesthetized with 25 mg of pentobarbital sodium/kg iv. The first group was administered 20-mg doses of levodopa[1] into their brachial vein over 30 sec. After the dogs in the second group were fixed on their backs, a laparotomy was performed and levodopa solution[1] was administered directly into the hepatoportal veins over 30 sec. A laparotomy was performed in the third group, a 20-cm segment of the duodenum was ligated, and levodopa solution was administered into the ligated loop.

Blood samples were withdrawn from each animal with a heparinized syringe from the femoral or brachial vein at the time intervals indicated in Fig. 1. The blood specimens obtained were processed as described previously (1, 3). The third group was killed by exsanguination immediately after the last blood sample was collected, and the ligated duodenal loops were removed to determine residual levodopa and its metabolites in the duodenal loops where levodopa was administered. The contents of the duodenal loops were washed with saline and then three times with 0.04 *N* $HClO_4$ solution. The irrigating solutions were assayed for residual levodopa and its metabolites.

Influence of GI Microorganisms on Oral Levodopa Absorption—Two healthy male mongrel dogs, 6.0 and 11.8 kg, were orally administered a single capsule containing 110 mg of paromomycin and 100 mg of kanamycin. Two control dogs also were used. The dogs were anesthetized with intravenous injection of 25 mg of pentobarbital sodium/kg. After anesthetization, a laparotomy was performed and a series of intestinal loops were made by ligating segments of the stomach, duodenum, and jejunum. The contents of each segment were suspended in saline solution and diluted with 0.1% phosphate buffer (pH 7.2). The

[1] Dopaston Injection, Sankyo Co., Tokyo, Japan.

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